Internal Medicine
The Japanese Society of Internal Medicine
doi: 10.2169/internalmedicine.4196-19
Intern Med 59: 1555-1558, 2020
http://internmed.jp

【 CASE REPORT 】

An Adult Case of Generalized Convulsions Caused by the Ingestion of *Ginkgo biloba* Seeds with Alcohol

Fumika Azuma[1], Kazuya Nokura[1], Tetsuharu Kako[1], Daisuke Kobayashi[2], Teruki Yoshimura[2] and Keiji Wada[2]

Abstract:
A 64-year-old woman developed symptoms of vomiting and tonic-clonic convulsions 9.5 h after eating 50 roasted *Ginkgo biloba* seeds with 100 g of alcohol. The intravenous administration of pyridoxal phosphate effectively improved the symptoms. Blood samples were collected and stored over 35 h. The assessment of 4'-O-methylpyridoxine and vitamin B6 vitamers indicated high levels of both, but the pyridoxal phosphate levels were low during the acute stage. These results suggest that 4'-O-methylpyridoxine inhibits the transformation of vitamin B6 analogues to the active form, pyridoxal phosphate. In our case, alcohol may have extended the period until ginkgo intoxication appeared.

Key words: *Ginkgo biloba* seeds poisoning, generalized convulsion, 4'-O-methylpyridoxine, pyridoxal phosphate, treatment, Ginkgotoxin

(Intern Med 59: 1555-1558, 2020)
(DOI: 10.2169/internalmedicine.4196-19)

Introduction

The consumption of *Ginkgo biloba* (GB) seeds (Gin-nan/Ginkyo in Japanese) is common in Japan, Korea, and China. In Japan, GB seeds are consumed as a seasonal treat in autumn, but vomiting and convulsion may appear when GB seeds are consumed in large quantities, especially in children. Wada et al. investigated GB seed poisoning by identifying the causal substances of the poisoning by first performing animal experiments, and then by further measuring the blood concentrations of patients known to have consumed GB seeds (1-3). The mortality rate is approximately 13% (22 of 170) in Japan, and no deaths caused by GB seed poisoning have been reported since 1969 (4). Of the cases of GB seed poisoning reported, many have been in children (3-10) and only a few in adults (11, 12), and few reports have been published in the English literature (13, 14).

The toxic component in GB seeds that causes convulsion has been identified as 4'-O-methylpyridoxine (MPN) and may inhibit the production of pyridoxal phosphate (PLP; active form of vitamin B6). In addition, because PLP is a coenzyme of glutamate decarboxylase, the formation of γ-aminobutyric acid (GABA) may be decreased, resulting in convulsion (2, 15, 16).

We recently used a rat model to examine the plasma concentration changes in B6 vitamers [PLP, pyridoxal (PL), and 4-pyridoxic acid (PA)] after intravenous MPN administration. Significant decreases in the concentration of PLP as well as an increase in the PL and PA concentrations were observed (17). We herein report a case of GB seed toxicity in which we examined the serum concentrations of these vitamin B6 analogues during intoxication.

Case Report

A 64-year-old woman with no significant medical history visited the emergency department with symptoms of vomiting and tonic-clonic convulsions. Neither she nor anyone in her family had ever had an epileptic episode. She has consumed distilled spirits (100-150 g alcohol) every day for over 40 years. From approximately 7:00 pm to 10:00 pm, she consumed around 100 g of alcohol while eating about

[1]Department of Neurology, Fujita Health University, Bantane Hospital, Japan and [2]School of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Japan
Received: November 15, 2019; Accepted: December 26, 2019; Advance Publication by J-STAGE: March 5, 2020
Correspondence to Dr. Kazuya Nokura, knokura@fujita-hu.ac.jp

Table. Serum Concentration of Each Substance.

A) (nM)						
time after ingestion	PLP	PL	PA	MPN	PLP/PL	PLP/PA
10 h	12	150	200	3,461	0.08	0.06
14 h (1 h after adm. of PLP)	12,311	4,116	2,304	3,230	3.0	5.3
35 h	22,693	3,335	1,344	<100	6.8	16.9
reference value*	21-138	5-26	9-60			
reference value**	19.8-200	4.2-24.5	6.1-107		2.3-13.7	

B) (ng/mL)				
time after ingestion	PLP	PL	PA	MPN
10 h	3.0	25	37	634
14 h (1 h after adm. of PLP)	3,042	688	422	592
35 h	5,607	558	246	<20
reference value*	5.2-34.1	0.8-4.3	1.6-11.0	
reference value**	4.9-49.4	0.7-4.1	1.1-19.6	

[A]units in nM,[B]units in ng/mL. Serum concentration of each substances at each point.

Reference values from the literature *(18), **(19).

PLP: pyridoxal phosphate, PL: pyridoxal, PA: pyridoxic acid, MPN: 4'-O-methylpyridoxine, adm.: administration

50 roasted GB seeds. At 5 h after she consumed the seeds (3:00 am), she began vomiting. She had the first convulsion at around 7:30 am, which was 9.5 h after ingesting the GB seeds. When she arrived at the emergency department, her symptoms had improved slightly. Emergency physicians considered acute alcohol intoxication and started her on intravenous fluids. However, at 9:05 am, the convulsion appeared again, and they administered an intravenous infusion of diazepam. The symptoms returned, and she was admitted to the hospital.

At admission, her height was 160 cm, her weight was 55 kg, blood pressure was 148/105 mmHg, pulse was regular at 66/min, and her temperature was 35.5℃. There were no other noteworthy findings. The pupils were round but reacted slowly to light. There was no obvious paralysis or sensory disorder. The deep tendon reflex was normal, but the plantar reflexes were extensor bilaterally. The patient was drowsy and disoriented. Although she was able to answer simple questions, she was unable to follow instructions completely. She displayed signs of irrational behavior, such as getting out of bed without permission.

On admission, an analysis of her arterial blood gases under oxygen administration (2 L/min) via nasal cannula showed mild acidosis (pH 7.245; pCO_2 at 46.9 mmHg; pO_2 at 133.0 mmHg, and HCO_3 at 19.9 mmol/L). Mild hepatic dysfunction was also noted. The liver function test showed a total bilirubin level of 1.4 mg/dL, AST 92 IU/L, ALT 69 IU/L, LDH 232 IU/L, γ-GTP 346 IU/L, and CK 61 IU/L. The remaining results from the blood screening tests were normal. Levels of vitamins B1, B2, and B12 as well as folic acid and ammonia were also within the normal range. Her inflammatory markers, kidney, electrolytes, and blood sugar were normal, and she was negative for any hepatic viral infections. The first serum sample was stored for 10 h after ingestion. She underwent computed tomography (CT) of the head and abdomen. Abdominal CT showed signs of a fatty liver, while head CT findings were normal. In addition, the cranial magnetic resonance imaging (MRI) and electroencephalogram findings obtained the next day were normal.

Immediately after admission, at 10:35 am, she had generalized tonic-clonic convulsions again for about 2 minutes. She started intravenous PLP (30 mg twice a day) for 2 days. An hour later, the next blood sample was taken (14 h after the last ingestion of GB seeds). There was no subsequent recurrence of convulsions. On the second day of hospitalization, her symptoms and abnormal neurological examination findings disappeared. On the third day of hospitalization, she was found to have completely recovered and discharged herself.

Measurement studies

The serum concentrations of PLP, PL, PA, and MPN were simultaneously measured by high-performance liquid chromatography with a fluorescence detector according to the previously reported method (17).

Measurements were conducted twice, and the mean values (n=2) are shown in the Table. At the arrival time (10 h after the last ingestion), MPN was >3,000 nM, which indicates GB intoxication. In contrast, PLP was relatively low, while PL and PA were relatively high. An hour after replacement of pyridoxal phosphate, the levels of PLP, PL, and PA were significantly increased. PL and PA decreased with time. Blood samples at 35 h after ingestion showed low levels of MPN. Reference values were obtained from the literature (18, 19).

Discussion

GB, one of the oldest living tree species in the world, has a long history in traditional Chinese medicine. Today, the

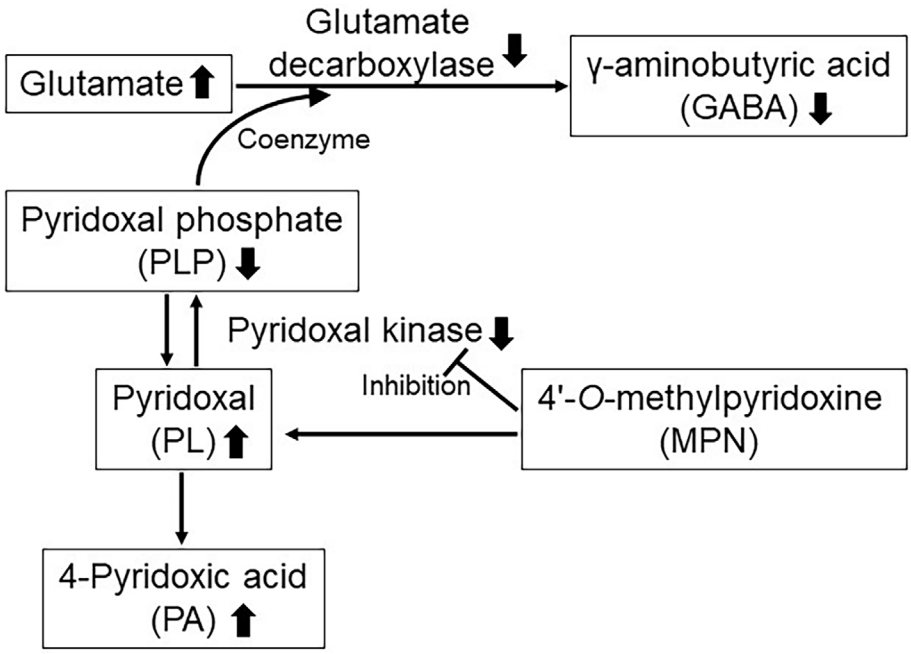

Figure. **The effect of MPN on vitamin B6 concentration and GABA synthesis (16, 17).**

extract from GB leaves is used as a dietary supplement for many conditions, including dementia (20) and other health problems (4). Interestingly, no conclusive evidence has shown that GB is helpful for any health condition (21).

GB seeds contain components of anti-vitamin B6 (2). Vitamin B6-dependent convulsions have been reported, especially in the pediatric field (22). Vitamin B6 may thus be closely related to convulsions. In addition, reports suggest that vitamin B6 levels are decreased in people with alcoholism (23). In most cases, the MPN concentration at the time of poisoning has been reported to be ≥500 nM (9), and our case showed an MPN concentration of 3,461 nM, suggesting acute poisoning of GB seeds. Serum MPN was not detectable the next day, and her symptoms also improved along with the disappearance of MPN from the serum. However, the concentration of PLP was 12 nM at poisoning, indicating acute deficiency of vitamin B6. Interestingly the concentrations of PL and PA were relatively high, but both the PLP/PL and PLP/PA ratios were extremely low, which was the same finding as seen in pharmacokinetic studies of MPN after intravenous (17) and oral administration to rat (24). These similarities suggest that MPN may inhibit pyridoxal phosphorylation by pyridoxal kinase in both rats and human (Figure).

In a previous report, the cerebrospinal fluid of epileptic patients showed lower PLP concentrations and PLP/PL ratios than that of non-epileptic patients (25), suggesting that susceptibility to epilepsy may be associated with these findings. In that same report, PL kinase was suggested to play an important role in the metabolism of vitamin B6 vitamers, especially in the brain. Even in cases with intoxication, the concentration ratio of each substance (MPN/PLP, PLP/PL, and PLP/PA) may be a more sensitive marker than the plasma PLP concentration. The detailed mechanism underlying MPN intoxication should be elucidated in future studies.

In many reports written in Japanese, symptoms have been reported to appear at 1 to 12 h after the ingestion of GB seeds, appearing in most cases by 6 h. However, children show an onset within several hours after ingestion, while convulsions started at 12 h in a 38-year-old woman (9), and symptoms appeared at approximately 12 h in a 51-year-old woman (14). In our case, the first convulsion occurred after a relatively long latent period, suggesting that a symptomatic latency period and prolongation are observed in adult cases.

The mechanisms leading to this relatively long latent period should be discussed. The adult brain is considered to have a higher threshold of seizure occurrence than a child's brain. Therefore, adults may require the ingestion of a greater amount of GB seeds to show seizures than children, and the blood levels of toxic substances after ingestion of GB seeds may rise more slowly in adults than in children. High concentrations of MPN were detected after a relative long latent period in our case, possibly due to alcohol consumption altering the pharmacokinetic parameters, such as by enhancing GB seed digestion and MPN absorption and inhibiting MPN metabolism. However, no studies have examined this hypothesis. The relationship between alcohol, seizure threshold, toxin intake, and the metabolisms of toxic substances are complex, and the role of alcohol consumption in this case is unknown. Alternatively, alcohol may have also prolonged the latency period, since this substance decreases the activity of excitatory transmission of glutamate and enhances GABAergic transmission (26).

Although diazepam and midazolam are effective for convulsions caused by GB poisoning, the recurrence of convulsions may occur. Symptoms disappear relatively quickly when vitamin B6 (PLP or pyridoxine) is administered. The

administration of vitamin B6 is the most effective treatment for food poisoning by GB seeds (5, 8, 9).

This is the first case report where the patient had actual convulsions due to GB poisoning, and the serum concentration of various B6 vitamers were measured over time. In order to measure MPN and B6 vitamers, it is necessary to collect and keep not only serum but also cerebrospinal fluid samples over appropriate time intervals. It is very important to note the history of ingestion of GB seeds so as not to confuse the patient as having alcoholism or epilepsy.

The authors state that they have no Conflict of Interest (COI).

References

1. Wada K. Data Base on Mechanism of Ginkgo Food Poisoning [Internet]. [cited 2019 Nov 14]. Available from: http://www.hoku-iryo-u.ac.jp/~wadakg/ginkgomecha.html
2. Wada K, Ishigaki S, Ueda K, Sakata M, Haga M. An antivitamin B6, 4'-methoxypyridoxine, from the seed of Ginkgo biloba L. Chem Pharm Bull (Tokyo) **33**: 3555-3557, 1985.
3. Yagi M, Wada K, Sakata M, Kokubo M, Haga M. [Studies on the constituents of edible and medicinal plants. IV. Determination of 4-O-methylpyridoxine in serum of the patient with gin-nan food poisoning]. Yakugaku Zasshi **113**: 596-599, 1993 (in Japanese).
4. Mei N, Guo X, Ren Z, Kobayashi D, Wada K, Guo L. Review of *Ginkgo biloba*-induced toxicity, from experimental studies to human case reports. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev **35**: 1-28, 2017.
5. Kajiyama Y, Fujii K, Takeuchi H, Manabe Y. Ginkgo seed poisoning. Pediatrics **109**: 325-327, 2002.
6. Wada K. [Ginkgo seed food poisoning]. Chudoku Kenkyu **18**: 11-16, 2005 (in Japanese).
7. Fujisawa M, Hori Y, Nakajima M, Shimada K, Yoshikawa H, Wada K. Gas chromatography-mass spectrometry analysis of 4-O-methylpyridoxine (MPN) in the serum of patients with ginkgo seed poisoning. J Anal Toxicol **26**: 138-143, 2002.
8. Hasegawa S, Oda Y, Ichiyama T, Hori Y, Furukawa S. Ginkgo nut intoxication in a 2-year-old male. Pediatr Neurol **35**: 275-276, 2006.
9. Hori Y, Fujisawa M, Shimada K, Oda A, Katsuyama S, Wada K. Rapid analysis of 4-O-methylpyridoxine in the serum of patients with *Ginkgo biloba* seed poisoning by ion-pair high-performance liquid chromatography. Biol Pharm Bull **27**: 486-491, 2004.
10. Lorenzo CD, Ceschi A, Colombo F, et al. Identification and quantification of biomarkers to confirm the poisoning by *Ginkgo biloba* seeds in a 2-year-old boy. Toxicol Res **4**: 922-930, 2015.
11. Irie H, Yamashita S, Shinjyo Y, et al. An adult case of Ginkgo seed poisoning with toxic substance detected in blood and cerebrospinal fluid. ICU & CCU **32**: 167-172, 2008.
12. Miyazaki D, Kubota T, Kobayashi D, et al. A case of Ginkgo seed poisoning in a healthy adult. J of Jpn Assoc Acute Med **21**: 956-960, 2010.
13. Miwa H, Iijima M, Tanaka S, Mizuno Y. Generalized convulsions after consuming a large amount of gingko nuts. Epilepsia **42**: 280-281, 2001.
14. Jang HS, Roh SY, Jeong EH, Kim BS, Sunwoo MK. Ginkgotoxin induced seizure caused by vitamin B6 deficiency. J Epilepsy Res **5**: 104-106, 2015.
15. Nitsch C, Okada Y. Differential decrease of GABA in the substantia nigra and other discrete regions of the rabbit brain during the preictal period of methoxypyridoxine-induced seizures. Brain Res **105**: 173-178, 1976.
16. Minami M, Yanai A, Endo T, et al. Convulsion induced by 4-O-methylpyridoxine from the seed of the *Ginkgo biloba* L., in guinea pigs and rats. Life Sci Adv **9**: 107-115, 1990.
17. Kobayashi D, Yoshimura T, Johno A, Ishikawa M, Sasaki K, Wada K. Decrease in pyridoxal-5'-phosphate concentration and increase in pyridoxal concentration in rat plasma by 4'-O-methylpyridoxine administration. Nutr Res **35**: 637-642, 2015.
18. Talwar D, Quasim T, McMillan DC, Kinsella J, Williamson C, O'Reilly DS. Pyridoxal phosphate decreases in plasma but not erythrocytes during systemic inflammatory response. Clin Chem **49**: 515-518, 2003.
19. Albersen M, Bosma M, Luykx JJ, et al. Vitamin B-6 vitamers in human plasma and cerebrospinal fluid. Am J Clin Nutr **100**: 587-592, 2014.
20. DeKosky ST, Williamson JD, Fitzpatrick AL, et al. *Ginkgo biloba* for prevention of dementia: a randomized controlled trial. JAMA **300**: 2253-2262, 2008.
21. NIH. Ginkgo [Internet]. [cited 2019 Nov 14]. Available from: https://www.nccih.nih.gov/health/ginkgo
22. Clayton PT. B6-responsive disorders: a model of vitamin dependency. J Inherit Metab Dis **29**: 317-326, 2006.
23. Majumdar SK, Shaw GK, O'Gorman P, Aps EJ, Offerman EL, Thomson AD. Blood vitamin status (B1, B2, B6, folic acid and B 12) in patients with alcoholic liver disease. Int J Vitam Nutr Res **52**: 266-271, 1982.
24. Qian Y, Su S, Wei M, et al. Interactions of pharmacokinetic profiles of Ginkgotoxin and Ginkgolic acids in rat plasma after oral administration. J Pharm Biomedi Anal **163**: 88-94, 2019.
25. Akiyama T, Akiyama M, Hayashi Y, et al. Measurement of pyridoxal 5'-phosphate, pyridoxal, and 4-pyridoxic acid in the cerebrospinal fluid of children. Clin Chim Acta **466**: 1-5, 2017.
26. Abrahao KP, Salinas AG, Lovinger DM. Alcohol and the brain: neuronal molecular targets, synapses, and circuits. Neuron **96**: 1223-1238, 2017.